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FISKERIDIREKTORATETS SKRIFTER
Serie Havundersøkelser
(*Report on Norwegian Fishery and Marine Investigations*)
Vol. XI No. 6
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COD POPULATIONS

Identified by a chemical method

By
EVA HENLY DANNEVIG

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PREFACE

In the study of the biology of fishes the scientist often meets the problem of «races» or «populations». This problem is regularly dealt with by studying differences in anatomy and biology.

During the last few years I have examined the proteins of fish-muscle. The results given in this paper indicate that the chemical method will be of value to the fishery biologists. To what degree the differences found are hereditary, is now being examined at this station.

The Flødevig Sea-Fish Hatchery,
May 1955.
Eva Henly Dannevig.

By studying the proteins from fish-muscle by means of paper partition chromatography we easily realize that different species give a different pattern on the chromatogram.

The chromatographic method is a rather new invention in biology. The paper partition chromatography was developed in 1944, and was in the following years used to a great extent in both medical and botanical biology, but the zoologists have till now neglected this new and very useful method. It has, on a very small scale, been used to study heredity in Drosophila, and has also been applied a little in taxonomic studies.

The method is very simple and rapid; it requires only small amounts of the material to be analysed, and the utensils necessary are usually present in all wellequipped laboratories. About 2 mm^3 of the pure muscle, taken from the dorso-lateral part of the newly caught fish, is squeezed on a strip of specially treated filterpaper. The spot is dried at room-temperature, and surplus material is removed. The paper is then hung in a chromatography chamber, one end dipping in a through containing the solvent (here,n-butanol:acetic acid: water and phenol:water). After a certain time, depending on solvent, grade of paper and temperature, the paper is removed from the chamber and dried at room-temperature. The paper-strip is then sprayed with a ninhydrin-solution and after drying, heated to 110° C for 5 minutes. There will now be some coloured spots on the paper, varying from red to purple, in a few cases a yellow spot may appear. Each spot represents an amino-acid containing compound. The compounds travel at different speeds, and are thus found at different distances from the starting point. The pattern on a chromatogram is thus dependent on the compounds present in the fresh fish-muscle, and the relative amount of each compound. The relative amounts of the different

compounds is compared by their colour-intensity, not their size. By comparing two chromatograms, two things are essential, the distance travelled relative to the distance that the water-front has moved, and the colour-intensity.

The classification of proteins might indicate that an albumine, one of the most common proteins, is something very definite, irrespective of its source. This is not so. We shall have to take «species specifity» into consideration. The albumine from human blood is not the same as that obtained from fish blood. These differences apply to all proteins. Before the development of the partition chromatography it was well-nigh imposible to illustrate these differences. Immunological tests, however, did show that such differences existed.

The proteins belong to a group of most complex chemical substances. They are essential constituents of protoplasm, and they provide amino-acids, some of which are essential food constituents. The proteins are characterized by the fact that on hydrolysis they yield from twenty to twenty-five different α — amino-acids. The differences between proteins are largely a matter of number, kind and the arrangement of such amino-acids within the protein molecule. Since the protein molecule is often built up of hundreds, and even thousands of these amino-acid-groups, the problem of determining protein structure is one of almost insuperable difficulty.

I am not here going to determine the different proteins present in the fish-muscle, I will just show how their specifity can be used to characterize different species, and some times also geographically separated populations of the same species.

The vertebrate character of the fishes is chemically demonstrated in at least two ways. First, all the fish-muscle so far studied contains creatine phosphate. Arginine phosphate is never present. Secondly, many fishes have been found to contain carnosine and anserine. These two aberrant dipeptides are known to occur widely, and perhaps universally, in the muscles of vertebrates, but, in spite of repeated attempts, have not so far been detected in invertebrates. The distribution of carnosine and anserine has attracted a good deal of attention, and reports in literature serve well to emphasize that there are group-specific and even perhaps species-spesific differences among the fishes. Clifford (1921), who studied the distribution of carnosine in the animal kingdom, stated her main conclusion in the following words: «The only relation brought out by this investigation is a morphological one. If the base is absent from one member of a zoological family, it appears to be absent from all.» Thus, among the fishes carnosine was not found in four flatfishes and two gadiformes

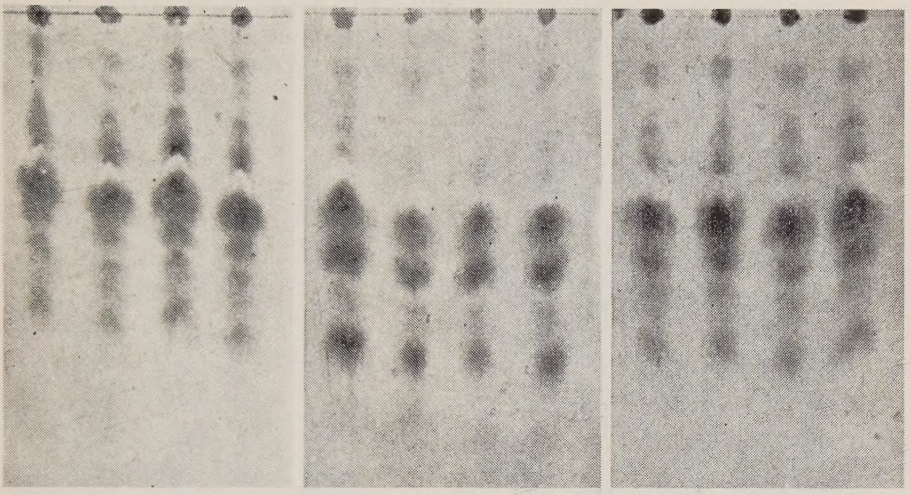

Fig. 1. From left to right: Chromatograms of Pleuronectes platessa, Pleuronectes microcephalus and a crossbreed between the two (Pl. platessa female and Pl. microchephalus male).

examined, while nineteen representatives of other suborders did contain it.

That there really do exist a species specificity between the proteins in the fish-muscle is demonstrated by chromatography of different species. Fig. 1 is a photograph of chromatograms of three flatfishes.

The chromatogram of the crossbreed gives a pattern in between those of the parents. If the press is able to give the differences in the intensity of the different spots, an examination of the 3 or 4 spots on the top of the chromatogram, will prove this.

Till now, 23 different species of marine fishes have been analysed, among these 10 Gadidae, 3 Clupeidae and 4 Pleuronectidae. They all show a high degree of species-specificity. (Dannevig 1955).

After having examined a great number of cod from several localities, however, I have found that there seems to exist a difference between different populations as to the relative quantities of the various amino-acid compounds present in the muscle. The main pattern is the same, but the relative amount of some of the constituents changes as we go from one locality to another.

Fig. 2 shows the chromatograms of cod from the Lofoten area (northern Norway) to the left, and from the Skagerack (southern Norway) to the right. The different spots on the chromatograms are encircled and enumerated 1 to 7. In the cod from the Lofoten spot

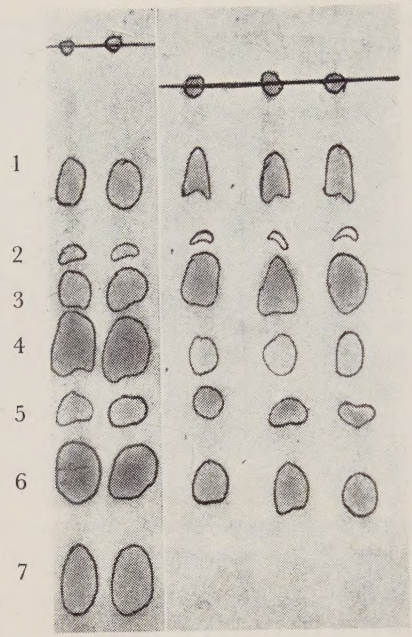

Fig. 2. Chromatograms of cod from the Lofoten area (northern Norway) to the left, and from the Skagerack (southern Norway) to the right.

No. 4 is much more intense than No. 3 and No. 5. In the cod from the Skagerack coast spot No. 4 is far weaker than those on either side. This character I have found to occur in all cod caught in the two areas, the Skagerack and the Lofoten — irrespective of local variations. Cod from deep waters in the Skagerack may, however, give a chromatogram where spot No. 4 is equally intense as No. 3, in other words approaches the chromatograms of the cod from Lofoten. According to Dr. A. Dannevig, this type of cod lives in deep and cold water, about 6° C.

When studying the chromatograms it is essential to be aware of the fact that it is the colour-intensity of the spot, and not the size that indicates the amount present of the compound. I hope the press will be able to give the differences in intensity).

But even cod caught within the same main locality can be separated in sub-groups.

Southern waters (Skagerack):

1. *Cod living in the Norwegian littoral zone.* The characteristic features are that they lack spots No. 2 and No. 7.

2. *Cod living in local deeper waters in the Norwegian «skjærgård».* This type is also sometimes found among the cod from shallow waters, but only as single specimens. Lacks spot No. 7.

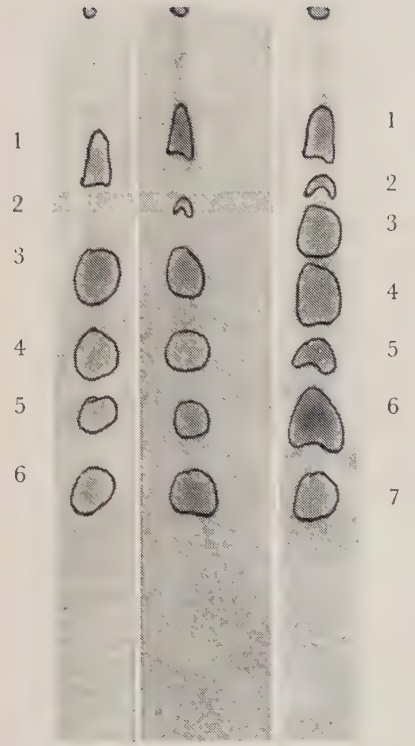

Fig. 3. Chromatograms of cod from Skagerack. From left to right are sub-groups 1, 2 and 3.

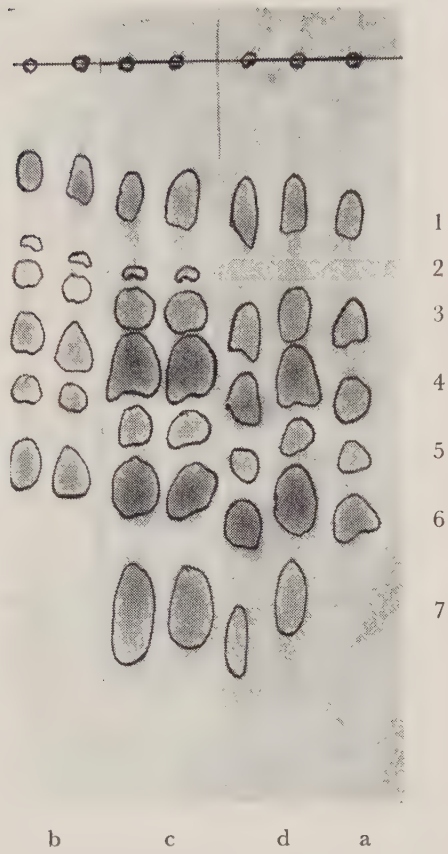

Fig. 4. Chromatograms of cod from the Lofoten area. From left to right: Sub-groups b, c, d and a.

3. *Cod from the open Skagerack.* Here all the seven spots are present. Spot No. 7 is getting intenser the deeper the cod is caught.

Fig. 3 gives a picture of the three different sub-groups from the Skagerack coast.

Northern waters (Lofoten area).

By analysing some samples from the spawning-area in the Lofoten, sent to me by courtesy of Gunnar Dannevig, I was able to separate the cod in the following sub-groups (The materiale from this area is, however, scarce):

a. *Resembles Skagerack-group No. 1,* Spots No. 2, and No. 7 are not present.

b. *Resembles Skagerack-group No. 2.* Lacks spot No. 7.

c. *Resembles Skagerack-group No. 3.* All the seven spots are present.

d. *This type is not yet observed in the Skagerack.* Lacks spot No. 2.

Mr. Rollefsen was kind enough to examine the otoliths from the same individuals, and, according to the charateristics of the zones, distinguished following types (His results and mine are given together in the table below):

No.	length	Age[1]	Type	Chromatography group
1	93 cm	8 (0)	Not characteristic. Bank cod? Murm. cod?	d
2	101 »	8—9 (0)	» »	d
3	74 »	7 (1)	Coastal cod	a
4	109 »	12 (1)	Not characteristic. Bank cod? Murm. cod?	d
5	99 »	12 (1—3)	Common skrei	c
6	95 »	9 (0)	Svaldbard-skrei	a
7	112 »	12 (2)	Common skrei	c
8	89 »	8 (0)	» »	c
9	105 »	13 (3)	» »	b

It is evident that the «skrei» corresponds to the cod from the open Skagerack. The coastal cod from Lofoten corresponds to the littoral cod in the southern waters. The individuals tentatively characterized as Bank cod? or Murmansk Cod? have not been observed in the Skagerack. Cod No. 9, by Rollefsen identified as «common skrei» gives a chromatogram corresponding to group b (which resembles Skagerack-group 2, cod from local deeper waters).

It is evident that the chromatography of muscle-fragments is satisfactory for identification of different fish-species and, it seems, even for different populations of cod. The characteristic features are caused by amino-acid-groups present in different quantities.

Some analyses reported in Biochemistry of Fish (1951) indicate that the composition of some of the proteins present in fish-muscle changes from one locality to another. Thus cod from European waters contain 0.35 % creatine (% of wet muscle) while cod from Canadian and American waters contain 0.576 %. Cod caught in the North Sea contain 0.33 % trimethylamineoxide, while those caught in the Arctic contain 0.55 %. The same probably occurs to other proteins present in the muscle. So in order to determine the differences between

[1] Nos in brackets indicate Nos of spawning-zones.

Fig. 5. Chromatogram of hydrolysed cod-muscle.

geographically separated populations of, for instance, cod it is necessary to determine the amino-acid composition of the hydrolysed muscle. This I have done for the cod from southern waters (Skagerack), and the results are given below, together with fig. 5, which shows the chromatogram of hydrolysed cod-muscle. (I have till now had no chance to analyse the hydrolysed muscle from cod caught in northern waters.)

As previously mentioned, these acids do not exist as free amino-acids in the muscle. They are constituents of the more or less complex built peptides and proteins that form the chromatographic pattern of the untreated muscle. Thus alanine and methylhistidine are surely combined to form the dipeptide anserine, while cystine, glutamic acid and glycine form glutathione, which previously has been reported to be present in cod-muscle. The volatile amines and tryptophane will not show up on the chromatogram of the hydrolysed muscle, as they are destroyed by the acid-hydrolyses. All the creatine present will be converted into creatinine, which will be found on the chromatogram of the untreated muscle.

Summary:

The chemical composition of the muscle of cod varies according to the different localities.

First we have a very definite difference in the chromatographic pattern of cod-muscle between cod from the southern waters (Skagerack) and those from northern waters (Lofoten area). This difference occurred in all specimens examined, irrespective of local varieties.

Secondly, the cod from the two localities can be separated in sub-groups.

Cod from the Skagerack:

1. Littoral cod.
2. Deep-sea cod from the Norwegian «skjærgård».
3. Cod from the open Skagerack.

Cod from the Lofoten area:

a. Coastal cod (resembles Skagerack-group 1).
b. Deep-sea cod (resembles Skagerack-group 2).
c. Common skrei (resembles Skagerack-group 3).

d. Type not characteristic. Bank cod? or Murm. cod. (This type has till now not been observed in the Skagerack).

Finally the cod muscle has been hydrolysed, and the different constituents building the complex molecules of the amino-acid-group containing part of the muscle are identified.

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